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Effects of Japanese Barberry (Ranunculales: Berberidaceae) Removal and Resulting Microclimatic Changes on *Ixodes scapularis* (Acari: Ixodidae) Abundances in Connecticut, USA

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ABSTRACT Japanese barberry (*Berberis thunbergii* de Candolle) is a thorny, perennial, exotic, invasive shrub that is well established throughout much of the eastern United States. It can form dense thickets that limit native herbaceous and woody regeneration, alter soil structure and function, and harbor increased blacklegged tick (*Ixodes scapularis* Say) populations. This study examined a potential causal mechanism for the link between Japanese barberry and blacklegged ticks to determine if eliminating Japanese barberry could reduce tick abundance and associated prevalence of *Borrelia burgdorferi* (Johnson, Schmid, Hyde, Steigerwalt, and Brenner). Japanese barberry was controlled at five study areas throughout Connecticut; adult ticks were sampled over three years. Each area had three habitat plots: areas where barberry was controlled, areas where barberry remained intact, and areas where barberry was minimal or absent. Sampled ticks were retained and tested for *B. burgdorferi* presence. At two study areas, temperature and relative humidity data loggers were deployed in each of the three habitat plots over two growing seasons. Intact barberry stands had 280 ± 51 *B. burgdorferi*-infected adult ticks/ha, which was significantly higher than for controlled (121 ± 17/ha) and no barberry (30 ± 10/ha) areas. Microclimatic conditions where Japanese barberry was controlled were similar to areas without barberry. Japanese barberry infestations are favorable habitat for ticks, as they provide a buffered microclimate that limits desiccation-induced tick mortality. Control of Japanese barberry reduced the number of ticks infected with *B. burgdorferi* by nearly 60% by reverting microclimatic conditions to those more typical of native northeastern forests.

KEY WORDS *Berberis thunbergii*, *Borrelia burgdorferi*, *Ixodes scapularis*, microclimate, vapor pressure deficit

Japanese barberry (*Berberis thunbergii* de Candolle) is a thorny, perennial shrub native to southern and central Japan (Ohwi 1965) that was first planted in North America in the late 1800s (Harrington et al. 2003). It has since escaped from landscape plantings and is now established in 31 states in the United States, the District of Columbia, and five Canadian provinces [U.S. Department of Agriculture (USDA), NRCS 2010]. Dense barberry stands are associated with a lack of desirable tree and herbaceous plant regeneration (Harrington et al. 2003). Barberry may alter nitrogen cycling, affecting soil biota (Kourtev et al. 1999, Ehrenfeld et al. 2001), as well as soil structure and function (Kourtev et al. 2003). Two Maine studies reported blacklegged tick (*Ixodes scapularis* Say) abundances were twice as numerous in exotic-invasive infested forests, particularly Japanese barberry, than in adjacent forests dominated by native shrubs (Lubelczyk et al. 2004, Elias et al. 2006). This is of concern as blacklegged ticks are the major vector for several agents that cause Lyme disease, human granulocytic anaplasmosis, and human babesiosis (Magnarelli et al. 2006). Japanese barberry management was documented to reduce both blacklegged tick abundance and infection prevalence with *Borrelia burgdorferi* (Johnson, Schmid, Hyde, Steigerwalt, and Brenner) in Connecticut (Williams et al. 2009). As a result, Japanese barberry infestations can have an indirect, adverse effect on human health.

Temperature and relative humidity (RH) are determining factors in the survival of blacklegged ticks because of their high surface area to volume ratio and resulting susceptibility to desiccation. Stafford (1994) maintained immature blacklegged ticks at constant temperature (27°C) while manipulating RH levels. Half of all ticks kept at 100, 93, 85, 75, and 65% RH died after 67.1, 26.6, 8.3, 1.3, and 1.1 d, respectively, suggesting that the critical threshold for tick survival is ≈80% RH. While these experimental variables were held constant in the laboratory, both temperature and RH exhibit daily fluctuations in the field. Bertrand and Wilson (1996) reported that blacklegged ticks in open

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fields suffered higher mortality rates than those in edge or forested habitats and that tick survival was negatively related to air temperature, vapor pressure deficit, and the coefficient of variation of RH. However, precipitation and temperature were reported to be poor predictors of annual abundances of nymphal blacklegged ticks (Schultze et al. 2009). Ostfeld et al. (2006) suggested that because ticks spend >95% of their lives on the forest floor digesting a blood meal, undergoing diapause, or questing, temperature and RH could have an impact on survival.

Our previous research documented that Japanese barberry is favorable habitat for blacklegged ticks, particularly where white-tailed deer (*Odocoileus virginianus* Zimmermann) populations are high and native shrubs are scarce, and that barberry management can reduce blacklegged tick density in such areas (Williams et al. 2009). In Williams et al. (2009), we speculated that the causal mechanism of this linkage was that the closed canopy growth form of Japanese barberry may better retain humidity, thus creating a more favorable microclimate for blacklegged ticks. We also noted that another contributing factor to the decline in tick abundance could have been the elimination of suitable questing habitat after the removal of Japanese barberry stems.

Our overall objectives of this research were to: (1) provide additional and longer-term data to further document the relationship between blacklegged tick abundance and Japanese barberry cover, (2) provide additional and longer-term data to show that Japanese barberry is a public health threat by harboring *B. burgdorferi*-infected blacklegged ticks, and whether controlling barberry would again reduce *B. burgdorferi* prevalence, and (3) determine if Japanese barberry provides suitable questing habitat and/or ideal microclimatic conditions, which then harbor increased abundances of *B. burgdorferi*-infected blacklegged ticks.

Materials and Methods

Study Areas. Five replicate study areas were established in geographically separate areas: two on South Central Connecticut Regional Water Authority property in the town of North Branford (Tommy Path and Tommy Top), two in the town of Redding on the Centennial Watershed State Forest, which is jointly managed by the Aquarion Water Company, The Nature Conservancy, and the Connecticut Department of Environmental Protection (Egypt and Green Bush), and one in northeastern Connecticut on the University of Connecticut Forest in Storrs (Storrs). All study areas had remnant stone walls running throughout and were once agricultural fields or pastures; Storrs and the North Branford study areas were abandoned in the early 1900s, as were the Redding study areas in the 1940s.

Forest management was negligible (fuelwood harvests of declining and subcanopy trees), except in North Branford where ≈70% of basal area was removed during a salvage harvest of eastern hemlock (*Tsuga canadensis* Linnaeus (Carrière)) in the early 1990s. The remaining upper canopy in North Branford was primarily sugar maple (*Acer saccharum* Marshall) with mixed oak (*Quercus* spp.), white ash (*Fraxinus americana* Linnaeus), American beech (*Fagus grandifolia* Ehrhart), and scattered yellow poplar (*Liriodendron tulipifera* Linnaeus). Upper canopies in Storrs and Redding were characterized by a predominance of white ash, red maple (*Acer rubrum* Linnaeus), oak, yellow poplar, and some cherry (*Prunus serotina* Ehrhart).

Because of low light conditions under intact upper canopies and browse damage caused by exceedingly high white-tailed deer populations (upwards of 40 deer/km^2), there were virtually no native shrub species on study sites except northern spicebush (*Lindera benzoin* Linnaeus) (Williams et al. 2009). In addition, there was little regeneration of native tree species and as a result, sightlines extended several hundred meters through the forest (Fig. 1). However, the invasive wine raspberry (*Rubus phoenicolasius* Maximowicz), multiflora rose (*Rosa multiflora* Thunberg), and burningbush (*Euonymus alatus* Thunberg) were also present in the understory. All study areas had medium to dense stands of mature Japanese barberry that dominated the understory and excluded desirable forest regeneration and native herbaceous vegetation (Fig. 1). Further details on stand histories, forest composition, soil types, and local climate can be found in Ward et al. (2009).

Plot Design and Japanese Barberry Control. The research consisted of two parallel studies. The first study was established in the winter of 2007, and all aboveground portions of Japanese barberry were mulched. Treatment details are given below. There were three study areas in this study: Egypt, Storrs, and Tommy Path. The second study was established in the winter of 2008 and Japanese barberry was left standing dead. There were two study areas in this study: Tommy Top and Green Bush.

Three habitat plots were established at each study area, which included an intact barberry infestation where barberry was not controlled (full barberry), an area where barberry was managed by a series of control methods (controlled barberry), and an area where barberry was naturally minimal or absent (no barberry). No barberry areas were located within the immediate vicinity and were similar in stand composition as the other two habitat types, but had very limited or no barberry in the understory. Where barberry was controlled at Egypt, Storrs, and Tommy Path, the habitat plot layout consisted of four contiguous 30 × 30 m subplots. At Tommy Top and Green Bush, plot layout consisted of eight 50 × 50 m subplots in a 2 × 4 grid. Barberry cover was estimated at all locations by sampling 100 0.5 m^2 areas within each habitat plot. The 0.5 m^2 sampling instrument consisted of a 4 × 4 grid with which percent barberry cover was determined by presence/absence within each cell.

Initial control of barberry at Egypt, Storrs, and Tommy Path was accomplished by mechanical cutting and shredding of the above-ground portion of the

Fig. 1. Japanese barberry infestation in Redding, CT. Author pictured is 2.0 m tall.

plant and was completed in March 2007. We used a hydraulically driven rotary wood shredder (model# BH74FM, Bull Hog, Fecon Inc., Lebanon, OH) mounted to a compact track loader (model# T300, Bobcat, West Fargo, ND) for initial control. Barberry clumps missed by the wood shredder (adjacent to trees, stone walls, or large rocks) were hand cut. Follow-up methods used to control new ramets (sprouts) were: directed flame with a 100,000 BTU backpack propane torch (model# BP 223 C Weed Dragon, Flame Engineering, Inc., LaCrosse, KS), foliar application of glyphosate, and foliar application of triclopyr. Follow-up control methods were applied separately on sub-plots within habitat plots, but for the purposes of this study, the entire habitat plot with multiple control methods were considered a single "managed" habitat. Follow-up control methods were completed in late June 2007. More details on specific control methods can be found in Ward et al. (2009).

Barberry was controlled on the Tommy Top and Green Bush study areas using only 400,000 BTU backpack propane torches (model# BP 2512 C Red Dragon, Flame Engineering, Inc.). During late winter and early spring 2008, the bases of individual barberry plants were flame treated until the stems became carbonized and glowed. Follow-up flame treatments of new ramets were completed in the summer of 2008.

As noted above, the two control treatments differed in that individual barberry clumps remained standing dead (referred hereafter as "standing dead") at both Green Bush and Tommy Top, whereas barberry plants were mulched to ground level at Egypt, Storrs, and Tommy Path (referred hereafter as "mulched").

Tick Sampling. Adult blacklegged ticks were sampled in all habitats at all study areas using standard flagging techniques when ticks were active in early spring and late autumn (Stafford 2007). A 1 m^2 piece of white canvas duck cloth attached to a dowel was used to flag vegetation or the forest floor over linear transects totaling 200 m in each habitat plot at Tommy Path, Egypt, and Storrs. Linear transects totaling 400 m were flagged in all habitat plots at both Green Bush and Tommy Top. Flags were checked for ticks approximately every 15–20 m. Gathered ticks were transported to a laboratory, stored in a hydrator, and incubated at 10°C. Sampled blacklegged tick densities (ticks/hectare) were calculated for each plot, within each study area, for each year of the study.

Adult blacklegged ticks were sampled each at Egypt, Storrs, and Tommy Path on five occasions in the fall 2007/spring 2008 and on four occasions in the fall of 2008. In the fall of 2009, Egypt and Tommy Path were each sampled on six occasions and Storrs was sampled on seven occasions. Green Bush and Tommy Top were each sampled on seven occasions in the fall of 2008 and on six occasions in the fall of 2009.

A three-factor (treatment–mulched versus standing dead; habitat type–full, controlled, none; year) analysis of variance (ANOVA) with study areas as replicates was used to examine the influence of treatment and habitat on adult tick densities. Tukey honestly significant difference (HSD) was used to maintain alpha levels at $P < 0.05$ for multiple comparison tests of differences between treatments, habitats, and years. In addition, Pearson correlation coefficient (r^2) was used to examine the relationship between adult blacklegged tick density and percent barberry cover.

Indirect Fluorescent Antibody (IFA) Staining Methods. All flagged ticks were retained for testing for the presence of *B. burgdorferi* spirochetes. A total of 1,180 adult blacklegged ticks that survived incubation were tested by using IFA techniques for *B. burgdorferi* presence: 108, 180, and 191 in 2007, 2008, and 2009, respectively, from the mowed treatment plots, and 370 and 331 for 2008 and 2009, respectively, for standing dead treatment plots. Tick midguts were dissected under a stereo microscope and contents were smeared onto 12 well glass microscope slides (#30–103HTC,

Thermo Fisher, Portsmouth, NH). *B. burgdorferi* spirochetes were identified in midgut contents by using IFA staining methods with monoclonal antibody H5332, which is specific for outer surface protein A of *B. burgdorferi* (Magnarelli et al. 1994). Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulins (KPL, Inc., Gaithersburg, MD) were diluted to 1:40 in phosphate-buffered saline solution and used as the second antibody. Infection status was determined by noting the presence of fluorescing spirochetes through a microscope under a mercury vapor lamp. Procedural details followed established protocols (Anderson et al. 1991, Magnarelli et al. 1994, 1997). All ticks that were alive after incubation were destroyed during this procedure.

Once tick analyses were completed, percentage of blacklegged ticks infected with *B. burgdorferi*, that is, prevalence of infection, could be determined for each treatment, habitat, and year combination. A three-factor (treatment–mulched versus standing dead; habitat type–full, controlled, none; year) ANOVA with study areas as replicates was used to examine the influence of treatment and habitat on *B. burgdorferi* infection prevalence. All *B. burgdorferi* infection prevalence values were arcsine transformed before analysis (Zar 1974). Tukey HSD was used to maintain alpha levels at $P < 0.05$ for multiple comparison tests of differences between treatments and among habitats and years.

Prevalence of *B. burgdorferi*-Infected Ticks. The percentage of ticks that tested positive for *B. burgdorferi* was multiplied by the observed adult blacklegged tick density to estimate the density of the *B. burgdorferi*-infected ticks. A three-factor (treatment–mulched versus standing dead; habitat type–full, controlled, none; year) ANOVA with study areas as replicates was used to examine the influence of treatment and habitat on *B. burgdorferi*-infected blacklegged tick densities. Tukey HSD was used to maintain alpha levels at $P < 0.05$ for multiple comparison tests of differences between treatments, habitats, and years. Pearson correlation coefficient (r^2) was used to examine the relationship between *B. burgdorferi*-infected blacklegged tick densities and percent live Japanese barberry cover.

Temperature and RH. Microclimate stations were deployed in each of the three habitat plots at Tommy Top and Green Bush from 6 June 2008 to 7 January 2009 and from 1 May to 9 December 2009. Each microclimate station had two data loggers (Hobo Pro Series, Onset Computer Corp., Bourne, MA) mounted to a metal stake; one at 0.3 m (ground level) and one at 1.0 m above ground (mid-canopy). At both study areas, each habitat had two randomly located microclimate stations. Data loggers were programmed to record temperature (°C) and RH every 30 min. Data were downloaded and imported to Excel (Microsoft Corp., Redmond, WA).

Temperature and relative humidity data were averaged daily for both study areas by habitat for both years. In addition, daily temperature and relative humidity ranges (the difference in daily maximum and minimum values) were averaged for both study areas by habitat type and height (ground level, mid-canopy) for both years.

We calculated vapor pressure deficit (VPD) using data from the microclimate stations. Vapor pressure deficit is defined as the difference in water vapor pressure in the air compared with the water vapor pressure when air is saturated at that temperature. VPD = saturated vapor pressure–vapor pressure. Relative humidity expresses the vapor pressure deficit as a fraction of saturation vapor pressure. RH% = (vapor pressure/saturated vapor pressure)*100. If all other factors are similar, the rate of evaporation of water increases with increasing VPD. High VPD values have been shown to be strongly related to tick mortality (Wilson et al. 1993). A relatively simple formula for calculating saturated vapor pressure of water as a function of temperature from World Meteorological Organization (WMO, 2008) is:

$$e_w = (6.112\ e^{(17.62\ t(243.12 + t))})100$$

with t in (° C) and e_w in (Pa).

Half-hourly VPD values from both Tommy Top and Green Bush were combined and then averaged by time and month, taking into account height above ground and habitat type, returning 48 mean VPD values (half hourly over 24 h) for each month for each habitat/height combination for both the 2008 and 2009 data sets. One-way ANOVA was used to determine differences in VPD between habitats by month for mid-canopy and ground-level data logger placement for 2008 and ground-level data logger placement for 2009. One-way ANOVA was also used to detect differences in mean daily temperature, mean daily RH, and daily temperature and RH ranges for each habitat for each year both logger placements. Tukey HSD was used to maintain alpha levels at $P < 0.05$ for multiple comparison tests of differences between habitat types.

Results

Japanese Barberry Control. Virtually all cut and shredded Japanese barberry clumps produced new ramets after the initial mechanical control on the Egypt, Storrs, and Tommy Path study areas. While the initial control did not kill barberry, mechanical control was successful in removing the above ground portion of barberry clumps. All follow-up control methods resulted in both increased mortality of barberry genets (the entire plant) and smaller genet size (Ward et al. 2010). Similar to mechanical control, nearly all flame treated barberry clumps produced new ramets after initial treatment at Green Bush and Tommy Top. However, regenerating ramets were generally smaller than those that grew from the root base of shredded clumps. Follow-up flame treatments also killed most new ramets, though the majority of dead ramets remained standing through winter 2008/2009 and were observed to be more brittle and beginning to topple in summer 2009. Drought-like conditions in late summer 2008 resulted in some mortality of already stressed barberry ramets at the Storrs study area. Japanese

Table 1. Mean (standard error) Japanese barberry cover (%) by treatment, year, and habitat type

Treatment	Year	Habitat type		No barberry
		Full barberry	Controlled barberry	
Mowed	2007	44% (11%)	3% (2%)	3% (1%)
	2008	44% (10%)	3% (1%)	3% (1%)
	2009	41% (14%)	2% (1%)	2% (2%)
	All years	43% (6%)	3% (1%)	2% (1%)
Standing	2008	51% (17%)	1% (0%)	1% (1%)
Dead	2009	66% (8%)	2% (1%)	2% (0%)
	All years	58% (9%)	1% (1%)	2% (0%)

barberry cover estimates for all treatments are shown in Table 1.

Tick Sampling. A total of 1,562 adult blacklegged ticks (795 male, 767 female) were collected. Mean tick densities were consistently greater in full barberry areas for all years (Table 2). Habitat had a significant effect on adult tick density ($F = 15.9$; df = 2, 33; $P < 0.001$). Adult tick densities in full barberry plots were significantly different from controlled barberry and no barberry plots (Fig. 2). In addition, adult tick density was positively correlated with percent coverage of live Japanese barberry ($r^2 = 0.51$; $P < 0.001$). Tick densities did not differ between plots that were mowed and plots that had standing dead barberry ($F = 0.007$; df = 1,33; $P = 0.93$).

Indirect Fluorescent *B. burgdorferi* Antibody Detection. Prevalence of infection was highly variable; ranging from 17 to 68% (Table 3). Habitat ($F = 6.1$; df = 2, 32; $P = 0.006$) and year ($F = 5.9$; df = 2, 32; $P = 0.007$) had significant effects on *B. burgdorferi* infection prevalence. *B. burgdorferi* infection in adult ticks in the no barberry habitat type differed significantly from other habitats (Fig. 3).

Prevalence of *B. burgdorferi*-infected Ticks. Density of *B. burgdorferi*-infected ticks differed among habitat types ($F = 15.2$; df = 2, 33; $P < 0.001$; Table 4). As full barberry plots had the highest adult tick densities (Fig. 2) and high infection prevalence (Fig. 3), it was not surprising that full barberry plots also had the highest density of *B. burgdorferi*-infected ticks. Because habitat type had an effect on both prevalence of infection and adult tick densities, the density of *B. burgdorferi*-infected ticks in full barberry was nine times that in areas without barberry (Fig. 4). Relative to full barberry plots, density of *B. burgdorferi*-infected ticks was reduced by nearly 60% on plots where Japanese barberry was controlled. Density of *B. burgdorferi*-infected ticks did not differ between plots that were mowed and plots that had standing dead barberry ($F = 0.002$; df = 1,33; $P = 0.96$).

Table 2. Mean (standard error) of sampled adult blacklegged tick densities (ticks/ha) by treatment, year, and habitat type

Treatment	Year	Habitat type		No barberry
		Full barberry	Controlled barberry	
Mowed	2007	737 (310)	253 (82)	77 (23)
	2008	600 (220)	146 (62)	108 (90)
	2009	374 (90)	226 (35)	86 (36)
	All years	570 (124)	208 (35)	90 (29)
Standing	2008	364 (39)	248 (48)	73 (48)
Dead	2009	439 (106)	294 (111)	83 (42)
	All years	402 (51)	271 (51)	78 (26)

Temperature, RH, and VPD. Data from the microclimate stations were analyzed for the periods of 6 June to 9 December for both 2008 and 2009, the interval for which data were collected for both years. Several of the data loggers malfunctioned and returned either no data or impossible/nonsensical information. In 2009, both mid-canopy data loggers failed in the no barberry habitat at Green Bush. Means for each habitat type were determined using data from at least one data logger/height above ground/study area combination. As a result, only data from the lower data loggers could be used for all habitat types in both study areas for 2009.

Mean daily temperature did not differ among habitat types in 2008 for the ground-level data loggers ($F = 0.078$; df = 2, 558; $P = 0.925$) or mid-canopy data loggers ($F = 0.222$; df = 2, 558; $P = 0.801$). Mean daily temperatures did not differ among habitats in 2009 for the ground-level data loggers ($F = 0.241$; df = 2, 558; $P = 0.786$).

Mean daily RH differed among habitats in 2008 for the ground-level data loggers ($F = 20.462$; df = 2, 558; $P < 0.001$). Mean daily RH in full barberry plots ($91.0 \pm 0.57\%$) differed from both no barberry ($85.3 \pm 0.71\%$; $P < 0.001$) and controlled barberry ($86.9 \pm 0.65\%$; $P < 0.001$). Mean daily RH differed among habitats in 2008 for the mid-canopy data loggers ($F = 13.5$; df = 2, 558; $P < 0.001$). Full barberry ($89.8 \pm 0.62\%$) differed from both no barberry ($85.2 \pm 0.70\%$; $P < 0.001$) and controlled barberry ($85.9 \pm 0.70\%$; $P < 0.001$). Mean daily RH differed among habitats in 2009 for the ground-level data loggers ($F = 27.8$; df = 2, 558; $P < 0.001$). Full barberry ($94.6 \pm 0.44\%$) differed from both no barberry ($89.7 \pm 0.61\%$; $P < 0.001$) and controlled barberry ($89.5 \pm 0.59\%$; $P < 0.001$).

In 2008, mean daily temperature range differed among habitats for ground-level data loggers ($F = 11.0$; df = 2, 558; $P < 0.001$). Full barberry (12.8 ± 0.34°C) differed from both no barberry (11.4 ± 0.29°C; $P = 0.004$) and controlled barberry (10.8 ± 0.28°C; $P < 0.001$). Mean daily temperature range did not differ among habitats for mid-canopy data loggers in 2008 ($F = 0.343$; df = 2, 558; $P = 0.710$). For 2009, mean daily temperature range did not differ among habitats for ground-level data loggers ($F = 0.595$; df = 2, 558; $P = 0.552$).

In 2008, mean daily RH range did not differ among habitats for ground-level data loggers ($F = 2.01$; df = 2, 558; $P = 0.135$). Mean daily RH range differed among habitats for mid-canopy data loggers in 2008 ($F = 8.7$; df = 2, 558; $P < 0.001$). Full barberry ($34.6 \pm 1.31\%$) differed from both no barberry ($41.8 \pm 1.30\%$; $P < 0.001$) and controlled barberry ($40.0 \pm 1.25\%$; $P = 0.008$). For 2009, mean daily RH range did differ among habitats for ground-level data loggers ($F = 10.826$; df = 2, 558; $P < 0.001$). Mean daily RH range was lowest in full barberry ($27.1 \pm 1.16\%$), which

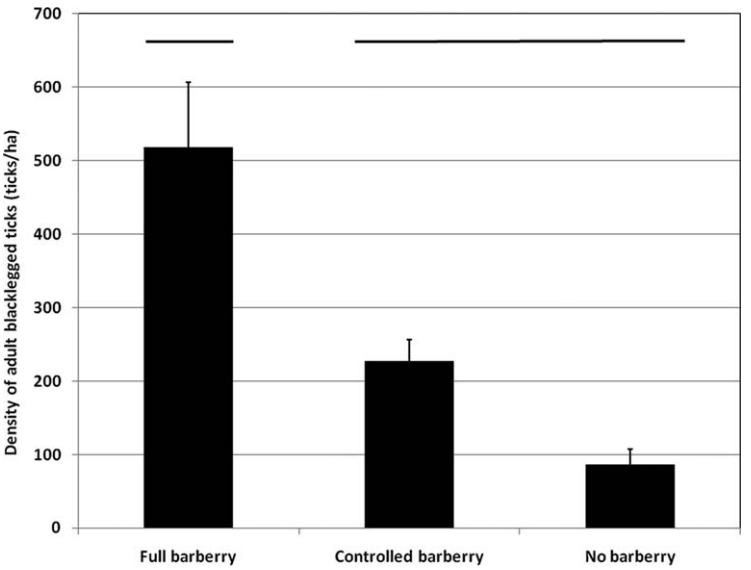

Fig. 2. Mean (standard error) of adult blacklegged tick densities (ticks/hectare) by habitat type. Habitat types linked by horizontal lines above bars were not found significantly different using Tukey's HSD test at $P < 0.05$.

differed from both no barberry (35.0 ± 1.29%; $P < 0.001$) and controlled barberry (32.2 ± 1.22%; $P = 0.009$).

Mean VPD values were consistently similar for no barberry and controlled barberry areas for all months for both ground-level and mid-canopy data logger placement for both 2008 and 2009 (Table 5). Ground-level VPD values were significantly lower in full barberry than no barberry areas for June through September 2008 and from June through October 2009. Mid-canopy VPD values were also significantly lower in full barberry than no barberry areas for July through September 2008. Results for comparisons between ground-level data loggers in full and controlled barberry areas were inconsistent between years. In 2008, mean VPD values were consistently lower in full barberry, but differences were only detected for August and September. In 2009, mean VPD values also were consistently lower in full barberry (Fig. 5) and differences were detected from June through October, which mimicked results when comparing no barberry to full barberry areas for that same year (Table 5).

Table 3. Mean (standard error) percentage of adult blacklegged ticks that tested positive for *Borrelia burgdorferi* by treatment, year, and habitat type

Treatment	Year	Habitat type		No barberry
		Full barberry	Controlled barberry	
Mowed	2007	44% (3%)	45% (5%)	17% (17%)
	2008	62% (6%)	52% (4%)	47% (3%)
	2009	50% (3%)	53% (6%)	34% (11%)
	All years	52% (3%)	50% (3%)	31% (8%)
Standing Dead	2008	60% (6%)	68% (1%)	61% (1%)
	2009	47% (1%)	53% (12%)	32% (22%)
	All years	53% (4%)	60% (6%)	47% (12%)

Discussion

The unforeseen consequences of accidental or intentional introductions of non-native species to ecosystems can have disastrous immediate and/or long-term impacts (Liebhold et al. 1995, Pimentel et al. 2005). While Japanese barberry is not directly detrimental to human health (other than its thorns), it does have an indirect negative impact to human health by harboring high densities of blacklegged ticks with high *B. burgdorferi* infection prevalence. Based on our results for the past three years (Fig. 4), there are an estimated 30 *B. burgdorferi*-infected adult blacklegged ticks/hectare in areas with no barberry and 280/ha in full barberry infestations. Barberry control efforts were successful in reverting microclimatic conditions to those similar of areas where barberry was absent. Concurrently with the change in microclimate, we observed a near 60% reduction in the number of *B. burgdorferi*-infected adult blacklegged ticks to 121/ha in areas where we controlled barberry (Table 4). We expect adult tick densities to continue to decline in areas where barberry was controlled as the 2-yr life cycle progresses with an assumed increase in desiccation-induced mortality at the larval and nymphal stages.

Temperature and RH data loggers proved invaluable in gaining an understanding of the microclimates in each of our habitat areas. Questing blacklegged ticks are susceptible to desiccation-induced mortality (Stafford 1994, Eisen et al. 2003, Schulze and Jordan 2003, Rodgers et al. 2007) and as a result, temperature, RH, and VPD are important variables when assessing tick habitats, associated tick densities, and Lyme disease risk (Schauber et al. 2005). Temperature is known to be positively correlated with blacklegged tick questing height and increased mobility of ticks,

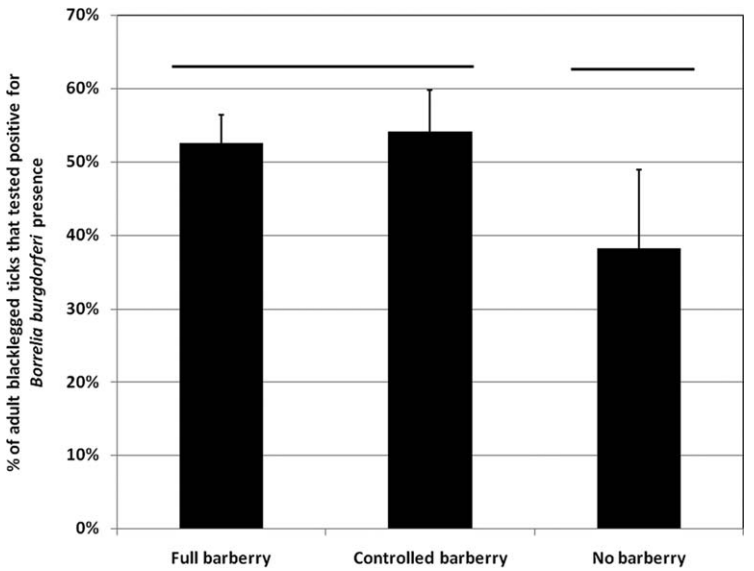

Fig. 3. Mean (standard error) percentage of adult blacklegged ticks that tested positive for *B. burgdorferi* by habitat type. Habitat types linked by horizontal lines above bars were not found significantly different using Tukey's HSD test at $P < 0.05$.

and this interaction was implicated in increased mortality of such ticks in a laboratory setting (Vail and Smith 2002). However, mean temperature did not differ between the habitats we examined in this study.

Calculating VPD includes both temperature and RH values and returns the "drying power" of air based on these values (Eaton and Kells 2009). Bertrand et al. (1996) found that blacklegged tick survival was lower in habitats with low RH and high VPD. Buffered microclimates without large swings in temperature and RH are desirable habitats for questing blacklegged ticks, limiting desiccation, and increasing questing time, that then increases the chances of finding a suitable host and surviving to the adult life stage (Sonenshine 1991). Our results show that Japanese barberry infestations provide such microclimatic conditions which are conducive to the survival of blacklegged ticks.

We believe that the data from the ground-level data loggers have more relevance for tick survival than the mid-canopy data loggers. The larval and nymphal life stages of blacklegged ticks are more prone to desiccation and tend to quest in vegetation closer to the ground when seeking their primary host, the white-footed mouse (*Peromyscus leucopus* Rafinesque) (Stafford 2007). We found that full barberry areas had consistently higher ground-level humidity and lower VPD than adjacent areas without barberry (Fig. 5). Mean monthly half-hourly VPD values were consistently lower in areas with intact barberry than in no barberry areas during months when plants retained leaves (Fig. 5).

Table 4. Mean (standard error) density (ticks/hectare) of *Borrelia burgdorferi*-infected adult blacklegged ticks by treatment, year, and habitat type

Treatment	Year	Habitat type		No barberry
		Full barberry	Controlled barberry	
Mowed	2007	342 (147)	108 (29)	7 (7)
	2008	399 (159)	74 (28)	48 (39)
	2009	189 (46)	119 (15)	23 (10)
	All years	310 (71)	100 (14)	26 (13)
Standing Dead	2008	221 (44)	168 (32)	45 (30)
	2009	204 (47)	168 (93)	36 (32)
	All years	212 (27)	168 (40)	41 (18)

In June and July, 2008, we observed that barberry plants were in the process of dying because of spring flame treatment, but the attached dead leaves aided in maintaining high humidity and low VPD mid-canopy. However, in 2009, both controlled barberry and no barberry areas had significantly higher mean VPD values than full barberry areas from June through October (Fig. 5). For both years, there was no difference in mean VPD values between controlled and no barberry areas. The mortality and subsequent leaf drop of Japanese barberry after flame treatment with propane torches was successful in converting the microclimatic conditions of a previously intact barberry stand to conditions similar to an area without barberry. This is the probable causal mechanism for the significant reduction in the blacklegged tick population and the density of blacklegged ticks infected with *B. burgdorferi.*

Eisen et al. (2003) stated that decreasing RH cannot only increase tick mortality, but also induce behavioral changes, such as ticks retreating to high humidity refugia. As a result, it is possible that because of their high and consistent daily RH levels and low VPD, Japanese barberry infestations effectively concentrate local blacklegged tick populations. We also found that by managing Japanese barberry infestations, we were able to reduce mean daily RH, mean daily temperature

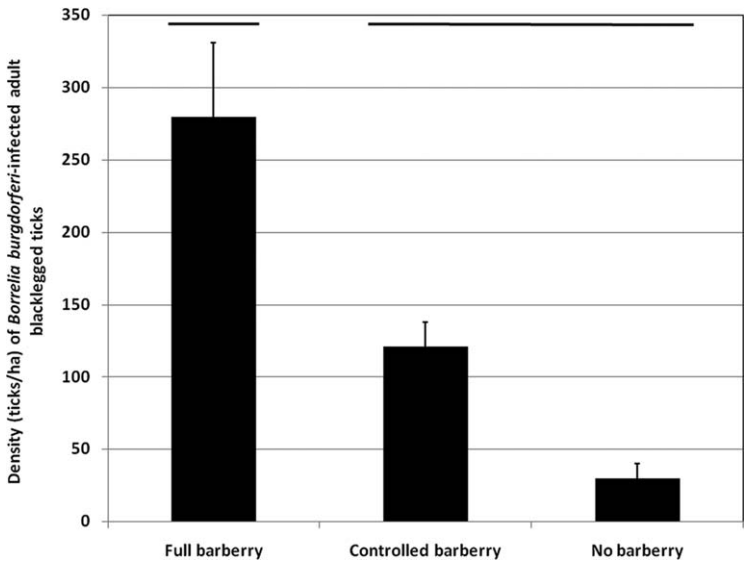

Fig. 4. Mean (standard error) density (ticks/hectare) of *B. burgdorferi*-infected blacklegged ticks by habitat type. Habitat types linked by horizontal lines above bars were not found significantly different using Tukey's HSD test at $P < 0.05$.

range, and resulting VPD to levels that were not different than adjacent areas without barberry. By returning the microclimatic conditions to this state, blacklegged tick populations were reduced in areas where Japanese barberry was managed. Additional evidence for this hypothesis was the observation that densities of *B. burgdorferi*-infected ticks did not differ in areas where barberry was left standing dead and areas where the aboveground portion of the plant was removed (Table 4). This evidence suggests that tick populations declined as a result of altered microclimate conditions and not because of the removal of questing habitat as we had previously suggested (Williams et al. 2009).

Because of their high surface area to volume ratios, larvae are the life stage most prone to desiccation (Stafford 1994, Subak 2003). Yoder and Spielman (1992) reported that larval blacklegged ticks better imbibe water from drier air than do nymphal or adult ticks, with a critical equilibrium humidity (CEH) of ≈85%. At RH below this CEH, larval ticks begin to desiccate. Larvae are most active in July and August in Connecticut (Stafford 2007), which is also when differences in VPD values were greatest between treatments (Fig. 5). Table 6 reports the number of successive hours/day that mean RH was below 85% for the months of July and August for ground-level data loggers for both 2008 and 2009. In 2009, mean RH in full barberry habitats was never below the CEH for July or August. Yoder and Spielman (1992) also reported that "Duration of questing, then, would be restricted to the interval that separates periods of rehydration, which presumably takes place on the more humid surface of the ground" (Loye and Lane 1988). Therefore, larval

Table 5. Reported *P* values from Tukey HSD tests for multiple comparisons for half-hourly mean monthly vapor pressure deficit values between full barberry (FB), no barberry (NB), and controlled barberry (CB) areas

Month	Year	Ground-level data loggers			Mid-canopy data loggers		
		FB-NB	FB-CB	NB-CB	FB-NB	FB-CB	NB-CB
June	2008	0.037*	0.150	0.823	0.096	0.150	0.976
July	2008	0.027*	0.065	0.939	0.027*	0.042*	0.986
Aug.	2008	0.001*	0.029*	0.612	0.008*	0.057	0.761
Sept.	2008	0.003*	0.029*	0.716	0.006*	0.066	0.673
Oct.	2008	0.067	0.178	0.895	0.109	0.271	0.885
Nov.	2008	0.378	0.770	0.797	0.946	0.592	0.788
Dec.	2008	0.102	0.282	0.860	0.256	0.402	0.955
June	2009	0.001*	<0.001*	0.981	—	—	—
July	2009	<0.001*	<0.001*	0.991	—	—	—
Aug.	2009	<0.001*	0.001*	0.921	—	—	—
Sept.	2009	0.012*	0.008*	0.994	—	—	—
Oct.	2009	0.006*	0.019*	0.922	—	—	—
Nov.	2009	0.128	0.248	0.937	—	—	—
Dec.	2009	0.056	0.063	0.999	—	—	—

Comparison that were significantly different for alpha levels at $P < 0.05$ are followed by an asterisk (*).

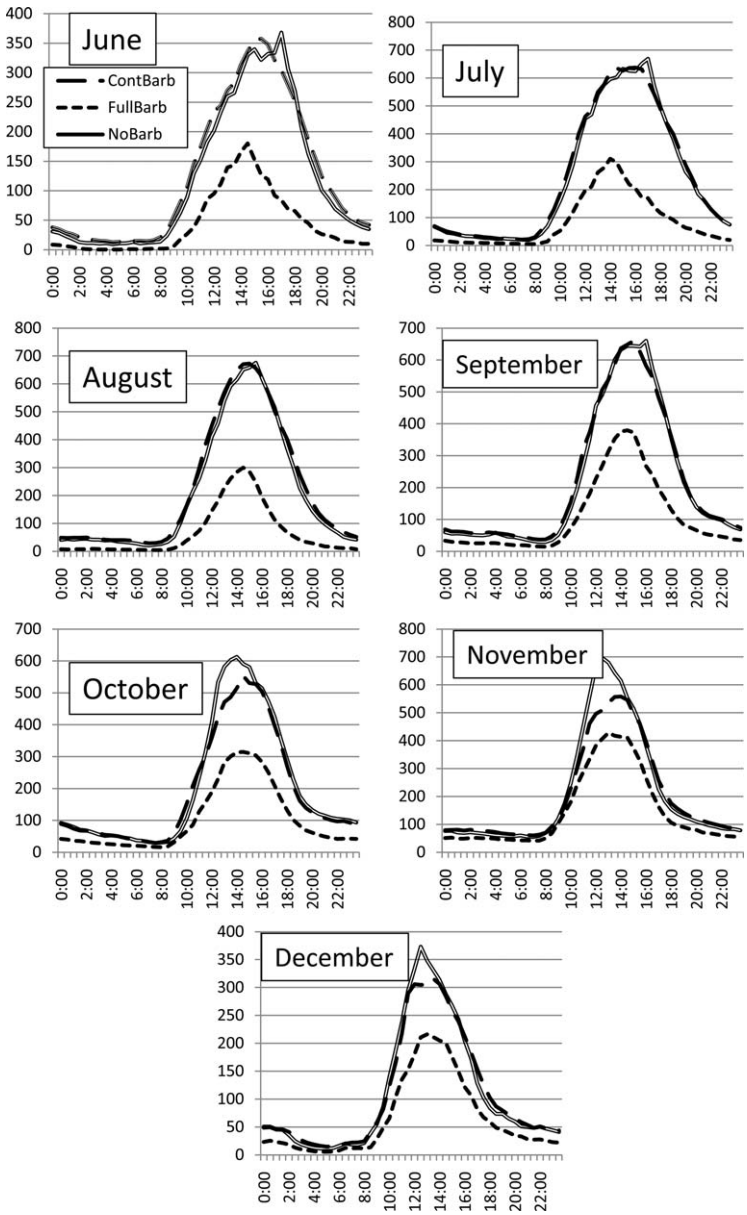

Fig. 5. Mean half hourly (x-axis) vapor pressure deficit values (Pascal, y-axis) by month for ground-level data loggers for full (FullBarb), controlled (ContBarb), and no (NoBarb) barberry areas for 2009.

ticks found in full barberry habitats are exposed to favorable microclimatic conditions that permit them to spend more time questing and less time retreating to higher RH refugia. Managing Japanese barberry likely increases larval mortality by creating a more hostile microclimate, thus reducing questing time and the chances of larvae obtaining a bloodmeal and surviving to the next life stage. Though there could have been limited immigration of ticks into barberry areas, we suspect that the higher adult blacklegged tick density was primarily because of the fact that barberry infestations are a favorable habitat for all life stages.

Subak (2003) found that Lyme disease incidence in humans was positively correlated with moisture levels, with some lag effects, throughout the northeastern United States because of increased tick survival. Stafford et al. (1998) found a positive correlation in Lyme disease cases and blacklegged tick abundances in Connecticut. Japanese barberry compounds this problem not only by enhancing local tick abundances, but doing so with blacklegged ticks with increased *B. burgdorferi* infection rates. In the interest of both forest and public health, we suggest that private landowners, land managers, and other land stewards with Japanese

Table 6. Number of successive hours/d that mean relative humidity was below 85% (the critical equilibrium humidity for larval ticks) for ground-level data loggers for July and Aug. 2008 and 2009 for full barberry (FullBarb), controlled barberry (ContBarb), and no barberry (NoBarb) habitat types

Month	FullBarb	ContBarb	NoBarb
Jul-08	3.5	8.0	8.0
Aug-08	2.0	7.5	8.5
Jul-09	0.0	7.5	6.5
Aug-09	0.0	4.5	3.5

barberry on their properties immediately initiate a management plan to address this alien invader.

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